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OBSERVATIONS ON MEDICINAL PEPSIN AND ARTIFICIAL DIGESTION.

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THE different preparations of medicinal pepsin are numerous and very extensively employed, but although previous experiments have shown that there exists a great difference in activity between the various brands, nothing is known certainly as to the relative power of each, nor is there any guide to the selection of the most effective. In 1857, some comparative observations upon three different sorts were made, and published by Dr. Sieveking, in the *Medical Times and Gazette;* in these experiments he employed coagulated egg-albumen, chopped finely; two of the preparations tried were of German make, and were found materially inferior in digestive power to the third used, which was Boudalt's. Subsequently, in 1863, Dr. Pavy made some further experiments with different preparations, comparing their action upon the legs of frogs; of those which he tried he found all inert, excepting only that of Messrs. Bullock and Reynolds; his results were published in the *Lancet* of that year. Afterwards, in 1870, Professor Tuson instituted and published [1] a long series of careful comparative experiments on the relative activity of six different preparations; the names of the makers of these, however, are not given, excepting that of Messrs. Bullock and Reynolds, whose pepsin was found to be very much more active than any of the others tried, one-half of which proved to be practically devoid of any proteolytic power.

These experiments sufficiently show the great difference

[1] *The Lancet*, 13 Aug. 1870.

which exists in the value, as a remedial agent, of the various preparations of pepsin, and the necessity for investigation. Having had occasion to employ some samples myself, I was desirous of ascertaining their relative activity, by testing them experimentally; the general results of this investigation are here published in the hope that they may be useful to others, who have occasion to employ this agent, and have not the opportunity of testing its activity themselves, and that a description of the methods used, and points which have occurred during the work, may assist those who shall engage in the same line of experiment.

The essential action of the gastric juice is proteolytic—the conversion of proteids into peptones; it also possesses the property of coagulating milk, precipitating its casein, and this, as has been shown, independently of any acidity; but these effects are probably the result of two distinct ferments, which coexist in the gastric juice, and which may be separated by suitable means; for by heating pepsin as usually prepared, its power of coagulating milk is destroyed, while its proteolytic action remains unimpaired; and when prepared by Brücke's method it does not coagulate milk, though an extremely active proteolyte. Whatever may be the value, from a therapeutical point of view, of its coagulating power, this must be a distinct question from that of its proteolytic action. Pepsin has no action upon starch; the gastric juice too, by its acidity, as is generally stated, hinders or destroys the amylolytic action of the saliva in the stomach, though Richet[1] asserts that the action of diastase on starch is increased in an acid medium. This however is a mistake, for I found that on adding equal small quantities of fresh saliva diluted and filtered[2] to two portions of starch paste, kept at 38° C., one of which was acidulated with 0·2 per cent. HCl, while in the neutral preparation the conversion of the starch, as indicated by Fehling's test, commenced very quickly, and was shortly completed, as shown by iodine; in the acidulated preparation, on the other hand, there was no trace of

[1] *Du Suc gastrique, &c.* Paris 1878, p. 116.

[2] I used fresh saliva as being much more active than any preparations of diastase that I could obtain; the action of which on starch was entirely prevented by 0·2 per cent. HCl.

sugar for some hours, and its complete transformation was indefinitely delayed. Pepsin has no action upon fat nor on either cane or grape sugar. It is generally said to hinder septic fermentation, but this, as remarked by Hoppe-Seyler and Richet, is a misstatement; the gastric juice is anti-septic, but it is so owing to its acidity, and not to any property of pepsin. I have found experimentally that if pepsin be added either to fresh urine or to hay infusion, it has no power to arrest septic fermentation and the development of bacteria, but, on the contrary, rather favours it; while if albumen be dissolved by pepsin, and the solution be then neutralised by Sod. Carb., it becomes most unmistakably septic and develops numerous organisms very rapidly, whereas if hay infusion be made acid by 0·02 per cent. HCl, fermentation is altogether prevented.

I have found that neither 1 per cent. of salicylic acid nor of sulphate of quinine appreciably interferes with the proteolytic action of pepsin. Borax is said by Dumas and by Schutzenberger to destroy the activity of all soluble ferments so decidedly as to serve as a test of whether one be of the soluble or of the organic class; but I have found that added in the proportion of 1 per cent. to the solution of pepsin, though it slightly retards its action, it does not prevent it.

The object of these experiments, then, was to test the proteolytic power of various preparations of medicinal pepsin. This may be done by different methods. First, by ascertaining the weight or quantity of each preparation requisite to dissolve completely a given weight of albumen or other proteid in a certain time; this is a convenient and sufficiently accurate method, and is the one here adopted. Some observers, as Bidder and Schmidt, have estimated the amount digested in a given time by weighing the residue, but in the case of coagulated albumen there is this inconvenience, that by maceration in acidulated water, it swells up, and increases in weight to the extent sometimes of 50 per cent., as I have ascertained experimentally. Others have estimated the amount of peptones contained in the solution, and this no doubt, in theory at least, is the most accurate method, but it is unnecessarily laborious for the object here in view. Another method, employed by Grützner, is a colorimetric one; the fibrine to be used is stained by carmine

and by comparing with a standard scale the coloration of the solution, the amount of fibrine dissolved is estimated. This with care would probably give reliable results, which might satisfy the observer, but would be less suitable for the information of others than the more definite statement of given weights and time. There are some other methods which may be used, but they are open to objections, from which the first above named is free, and which is that here employed.

I have examined fifteen different preparations of pepsin as named below, which[1] Mr. Martindale, of New Cavendish Street, was good enough to supply me with for this investigation ; I have generally used the same sample of each, excepting in the case of liquor pepticus (Benger), when, requiring a further supply, some of a different sample was received; this proved to be much more active than the first, and the result finally recorded is that afforded by this second sample.

I used coagulated egg-albumen in preference to fibrin, because it is more easily prepared in an uniform state of subdivision, and being wholly soluble without residue, the progress of digestion is readily watched and its completion ascertained. I found fibrin, either obtained from blood or the muscle of beef or mutton, very much less satisfactory to use. As far as my experiments went, I observed no difference in the relative activity of the various preparations, in their action upon fibrin, to that upon albumen, and as Professor Tuson has already shown (*loc. cit.*) that in the case of the preparations of pepsin which he examined "their relative digestive powers on fibrin are very nearly the same as those upon albumen," I did not think it necessary to experiment further in this direction. The state of subdivision of the substance to be digested very materially affects the rapidity of the process, and for comparative experiments it is in the highest degree requisite that this should be perfectly uniform. To effect this, the white of eggs previously hard boiled and cooled, freed from all membrane and yelk, was pressed through wire-gauze, in the first experiments, of about ten meshes to the inch, in the later, much finer, of about twenty-

[1] Excepting Wyeth's preparations, which were received from a friend to whom they had been sent by the proprietors to be tried.

four meshes to the inch;[1] this reduced the albumen to perfectly uniform grains or strips, not exceeding in diameter the size of the mesh. Of this the requisite quantity (in all the recorded experiments 100 grs.) was weighed out and placed in a small beaker. The beakers used were of similar size and shape; any difference in this respect might affect the results, for a preparation placed in a wide vessel offers a larger surface to the action of the solution, and also facilitates the diffusion through it of the products of digestion, thus accelerating solution in the same manner as constant agitation, or, more perfectly, the peristaltic movements of the stomach. The presence of air or oxygen, too, is essential to the action of the gastric ferment, and consequently the experiments should be conducted in vessels lightly covered. If air was excluded entirely, I found that though occasionally agitated the albumen was changed to a mass of caseous appearance and odour, which remained undissolved for an indefinite time, but upon admission of, and agitation with, atmospheric air, it was quickly dissolved. Frequent, or at least occasional, agitation of the preparation is requisite, for in examining some anomalies which occurred in the course of these experiments I found in some cases, though not apparently in all, that if coagulated albumen, in an acidulated solution of pepsin, be left perfectly undisturbed at a temperature of 38° C. for some hours, it formed an insoluble combination with a strong caseous odour and appearance,—very similar to that which is formed when air is excluded—which is not re-dissolved on the addition of fresh ferment, nor upon repeated agitation, but is so readily when fresh acid is added. This is more apt to occur, though not solely, in the case of the less active preparations, which require to be added in larger quantities. This result is obviously as important, in every point of view, as it was unexpected, not having been, as far as I am aware, noticed previously; and well merits farther investigation.

To the albumen thus prepared, an ounce of distilled water acidulated with 1 per cent. of hydrochloric acid of sp. gr. 1·150 (= 0·303 per cent. HCl) was added; this I found to be the most favourable degree of acidity for promoting digestion in

[1] I found that the coarser of the two preparations required fully half as long again for complete solution as the finer one did.

these experiments. If it deviated materially from this the result was much affected; if it were reduced one-half or increased to four times the quantity digestion was greatly retarded or wholly prevented. Most writers on artificial digestion state that 0·1 to 0·2 per cent. HCl, or less, is the degree of acidity most favourable to the digestion of albumen, fibrin requiring a smaller degree of acidity.[1] These results seem to be adopted from Bidder and Schmidt's analysis of the gastric juice or Brücke's experiments. Professor Tuson alone, as far as I have seen, states "that the result of special experiments indicates that this degree of acidity (1 per cent. by volume of concentrated hydrochloric acid) was more favourable to digestion than an acid of greater or less strength." Taking five preparations with a given quantity of albumen, weight of pepsin and volume of water in each, I acidulated them respectively with 0·1, 0·2, 0·3, 0·6, and 1·2 per cent. HCl, with the following results :—

No. 1.	0·1 per	cent. HCl	not digested in twenty-four hours.
„ 2.	0·2	„	not digested before eight hours.
„ 3.	0·3	„	digested in six hours.
„ 4.	0·6	„	not digested in ten hours.
„ 5.	1·2	„	not digested in twenty-four hours.[2]

Fibrin is said to require a smaller degree of acidity for its digestion than albumen. The quantity of water present too was found materially to affect the results; the weight of albumen and of the ferment used remaining the same, if the water was much increased or diminished the digestion was retarded. In one experiment where the quantity of water (in this case to five grms. albumen) was increased from thirty c.c. to ninety c.c., the digestion occupied double the time that it did with the first

[1] Statements as to the acidity of the gastric juice in man vary somewhat; no doubt it alters from different circumstances, as Manassein has shown in pyrexia artificially induced in dogs. The normal acidity in man is generally given at 0·2 per cent. HCl. Some, however, have found a greater degree of acidity, fully 0·3 per cent. (Hoppe-Seyler, *Physiol. Chem.* t. 2, s. 220). Richet (*Op. cit.* p. 91) gives some interesting observations, in a case of gastric fistula in man, on the effect of various substances ingested, upon the acidity of the gastric juice, and states that alkalies do not, as generally thought, increase its acidity, but that wine, which is always acid, does increase it, and brandy still more so.

[2] The degree of acidity was actually a fraction higher than that given above, *vide* p. 201.

Moleschott Untersuch. VIII. 1862. S. 325 etc.

Virchow. Archiv. f. pathol. Anat. &c. Bd. 55. 1872

quantity, and when it was reduced to fifteen c.c. it was indefinitely retarded.

The water having been added, the ferment, if in powder, was weighed out, and placed in a watch-glass on the beaker, which was labelled; when all was ready it was emptied into the beaker, which was then covered with the watch-glass and placed in the warm chamber at 38° C. (100°·4 F.). The warm chamber was such as is in general use in physiological laboratories, with glass sides and furnished with Page's regulator, by which the temperature can be kept perfectly constant without difficulty: this is a point of the very first importance in these experiments, for a difference of only a few degrees materially affects the activity of the ferment, and if disregarded will produce anomalous, and altogether unreliable results.[1] The temperature I employed was, as stated, 38° C. (100° F.), about that of the stomach in man, which is slightly increased during digestion (Ransome). Peptic digestion is promoted by an increase of temperature at least to several degrees above this point, up to, as is generally stated, about 50° C. (122° F.), above which it diminishes, and is stopped at 90° C. (191° F.). I have found that it is materially retarded by being lowered a few degrees only below 100° F., and in some of the earlier experiments, before this point was appreciated, it occasioned some trouble and anomalous results, but by the use of a Page's regulator there can be no difficulty in this respect: these details may seem trivial, but I have experienced their importance, and attention to them will save time and trouble to others who may institute similar experiments.

The following tables show the final results of this examination. No. I. shows the action of the maximum and minimum quality of each preparation recommended by the makers to be used; of the fifteen preparations tried nine were active in these quantities, and these, together with the second sample (B) of No. 2, which had been found to be much more active than the first, were then examined in order to ascertain the smallest quantities in which they were capable of dissolving the albumen employed as a test. The less energetic brands also were examined in the other direction, the quantity of each being

[1] Petit states that the same pepsin is four times less active at 40° than at 50°.

gradually increased, till, if practicable, it was found capable of effecting the solution. The results of these examinations are given in Table II., which also represents a series of experiments made simultaneously and repeated more than once, with the quantity therein specified of each preparation; an isolated experiment was seldom made, and never relied upon to establish any conclusion; the different preparations were always tried simultaneously under the same conditions, nearly always in duplicate, and the experiment repeated several times.

TABLE I.

Showing the result of the action in four hours at 100° *F. of different preparations of Pepsin, in the quantity specified, on* 100 *grs. of coagulated egg-albumen, in* 1 *oz. of dilute Hydrochloric Acid,* 1 *per cent.*

	Preparation.	Reaction.	Quantity used.	Result.
1	Glycerine of Pepsin (Bullock)	Acid	f. ʒ i.	All dissolved.
2	Liquor Pepticus (Benger) A. .	,,	f. ʒ i.	Not dissolved.
	,, ,, B. .	,,	f. ʒ i.	All dissolved.
3	Pepsin Wine (Morson) . .	,,	f. ʒ i.	Not dissolved.
4	Pepsin Essenz (Liebreich) .	,,	f. ʒ i.	,, ,,
	,, ,, ,, .	,,	f. ʒ iii.	,, ,,
5	Pepsina Porci B. P. (Bullock)	,,	grs. ii.	All dissolved.
6	Pepsin B. P. *ex ovibus* (Wright and Layman)	Faintly Acid	grs. ii.	Not dissolved.
	,, ,,	,,	grs. v.	All dissolved.
7	Pepsin B. P. *ex ovibus* (Hopkins and Williams) . . .	,,	grs. ii.	Not dissolved.
	,, ,,	,,	grs. v.	All dissolved.
8	Saccharated Pepsin (Finzelberg)	Neutral	grs. iii.	Not dissolved.
	,, ,, ,,	,,	grs. vi.	All dissolved.
9	Starch Pepsin (Boudault) . .	Acid	grs. v.	Not dissolved.
	,, ,, ,, . .	,,	grs. x.	All dissolved.
10	Pepsin Porci B. P. (Morson) .	,,	grs. ii.	Not dissolved.
	,, ,, ,,	,,	grs. iv.	All dissolved.
11	Starch Pepsin (Morson) . .	,,	grs. x.	,, ,,
12	Saccharated Pepsin (Wyeth) .	Neutral	grs. ii.	Not dissolved.
	,, ,, ,,	,,	grs. v.	,, ,,
13	Lactopeptine	Acid	grs. x.	,, ,,
	,,	,,	grs. xv.	,, ,,
14	Ingluvin	Faintly Acid	grs. v.	,, ,,
	,, ...	,,	grs. x.	,, ,,
15	Compressed Peptonic Tablet (Wyeth)	Acid	1 Tablet.	,, ,,

The above gives the minimum and maximum doses directed of each preparation.

No. 2 B was a second sample received of the same preparation, found to be much more active than the first (A); subsequent experiments were made with the second sample (B).

Nos. 8 and 9 appeared to be exactly the same make.

TABLE II.

Showing the results of experiments to ascertain the least quantities of the various preparations of Pepsin capable of completely digesting 100 *grs. of coagulated egg-albumen under the same conditions as in Table I.*

	Preparation.	Quantity.	Result.
1	Glycerine of Pepsin (Bullock)	f. ʒss.	All digested.
2	Liquor Pepticus (Benger) B.	f. ʒi.	,, ,,
3	Pepsina Porci (Bullock)	gr. ½.	,, ,,
4	Pepsin B. P. *ex ovibus* (Wright and Co.) .	grs. iv.	,, ,,
5	,, ,, (Hopkins and Co.) .	grs. iv.	,, ,,
6	Saccharated Pepsin (Finzelberg)	grs. v.	,, ,,
7	Pepsin Porci B. P. (Morson)	grs. iv.	,, ,,
8	Starch Pepsin (Boudault)	grs. vi.	,, ,,
9	,, ,, (Morson)	grs. x.	,, ,,
10	Lactopeptine	grs. xv.	Not dissolved.
11	Ingluvin	grs. xx.	,, ,,
12	Saccharated Pepsin (Wyeth)	grs. x.	,, ,,
13	Pepsin Wine (Morson)	f.ʒ iv.	,, ,,
14	Pepsin Essenz (Liebreich)	f.ʒ iv.	,, ,,
15	Compressed Peptonic Tablets (Wyeth) . .	4 Tablets.	,, ,,

The results of this examination, on the whole, *i.e.*, in the case of the large majority of the preparations, must be regarded as highly satisfactory; most of the brands show a high degree of energy, very small quantities dissolving in a few hours a large amount of albumen, though some few, as was anticipated, have failed. I have only to add that each experiment here recorded was repeated more than once, together with a very large number of others not recorded, the examination having been in fact a very long one, embracing twenty-three series containing upwards of 200 individual experiments and many more observations. The object proposed was solely to ascertain the *relative* proteolytic power of the different preparations; to this end each one in every series of experiments was submitted to identically the same conditions; the whole series of the final experiments gave precisely the same comparative results. Very imposing statements are sometimes made as to the absolute digestive capacity of this or that preparation; with such I have nothing to do; under slightly different circumstances as to temperature, the degree of acidity, dilution of liquid, frequency of agitation, &c., different results are certain to be obtained, but that will not

affect the question of the relative activity of the different preparations under the same conditions, with which alone I have dealt, and in this view only these results are offered.

POSTSCRIPT.—Since the above was written an article has appeared in the *Journ. de Pharm. et de Chimie* for January of this year (p. 82), by A. Petit (translated in the *Pharmaceutical Journal* of January 24th), on testing pepsine, which gives an excellent account, with many useful particulars of the process. He rejects the test by coagulation of milk, prefers that by fibrin to that by coagulated egg-albumen, states the effects of temperature, the comparative action of various acids, the most favourable degree of acidity, which, with hydrochloric acid, he finds to be 0·15 per cent. of real acid, conformably to the statements generally published; in other respects his conclusions, as far as comparable, correspond with my own, as given above. The degree of acidity, in the experiments in ~~Table I.~~, p. 197, was actually a fraction higher than that given there; for, to avoid the possibility of error, the strength of the acid employed was estimated gravimetrically by precipitation with Ag. NO_3. Its sp. gr. at 60° F. being 1·150, two, almost identical, determinations gave the percentage by weight of HCl as 30·30, which agrees exactly with the strength given in the tables of Davy, and is a little lower than that of Ure; the B. P. giving 31·8 for a solution of 1·16 sp. gr.